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Creation of a drug-sensitive reporter strain of *Pseudomonas aeruginosa* as a tool for the rapid screening of antimicrobial products

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ABSTRACT

Antibiotic resistance of bacteria is a considerable challenge to human health in the 21st century. With our discovery pipeline for new and effective antibiotics rapidly drying out, innovative approaches are needed to find new antimicrobials. Soil fungi are known to produce a variety of antimicrobials but rapid screening of fungi that produce such compounds remains a challenge. In this work, we used a hyper-susceptible strain of *Pseudomonas aeruginosa* to create a luminescent-reporter strain to be used as a screening tool to select fungi producing antimicrobials. We show that use of such a strain can not only significantly expedite the initial screening but also allows us to detect antimicrobials that may be produced in low concentrations. We believe that our reporter strain can be a valuable tool in identifying fungi that produce novel antimicrobials.

1. Introduction

Antibiotic resistance in bacteria is considered one of the leading threats to human health today. Antibiotic resistant bacterial infections are projected to become the most common cause of mortality in humans by the year 2050 (O'Neill, 2014). In particular, the situation is dire for Gram-negative bacteria for which there is no new antibiotics in the developmental pipeline (Boucher et al., 2017). As a result, the WHO has recently identified a list of 10 pathogens that pose a critical threat to humans because of their antibiotic resistance and for which treatment options are urgently needed (World Health Organization, 2017). One of the pathogens listed on the WHO list is *Pseudomonas aeruginosa,* a Gram-negative bacterium that causes a variety of infections in hospital settings, in immunocompromised individuals, and in burn patients (Roig and Sabria, 2003). *P. aeruginosa* has been shown to be one of the leading causes of hospital-acquired infections such as pneumonia, urinary tract infections, surgical site infections, and bloodstream infections. Its resistance to almost every single antibiotic in use makes the treatment of infections very challenging (Wright et al., 2017) and thus there is a critical need for new and effective antimicrobials to treat multidrug resistant *P. aeruginosa* infections.

Historically, a majority of antibiotics have been derived from natural products underscoring the potential of such compounds to be used as therapeutic agents. However, during the last few decades the strategy of finding natural products that contain antibacterial activity has achieved limited results (Demain, 2014). Nevertheless, it is believed that a large proportion of natural compounds with antibacterial activity remain undiscovered and innovative methods are needed to discover the potential of such compounds (Challinor and Bode, 2015; Katz and Baltz, 2016). Therefore, mining natural products remains a promising and valid option for the discovery of novel antimicrobials.

An important challenge in screening for new antibiotics is the activity of bacterial energy-dependent efflux pumps (Lomovskaya and Watkins, 2001). These pumps are capable of effluxing structurally unrelated compounds from inside the bacterial cell to the external environment and therefore a number of compounds with promising antibacterial activity can be missed in the screening process (Li et al., 2004). Current methods screen for antimicrobials by analyzing growth inhibition of target bacterial cells, which presents yet another challenge. These assays generally take 18–24 h, considerably limiting the ability to screen large numbers of compounds in a short period.

In this work, an efflux-deletion *P. aeruginosa* strain (Kumar et al., 2006) was modified by inserting the *lux* operon in single copy in its genome to allow for rapid high throughput screening for antimicrobial

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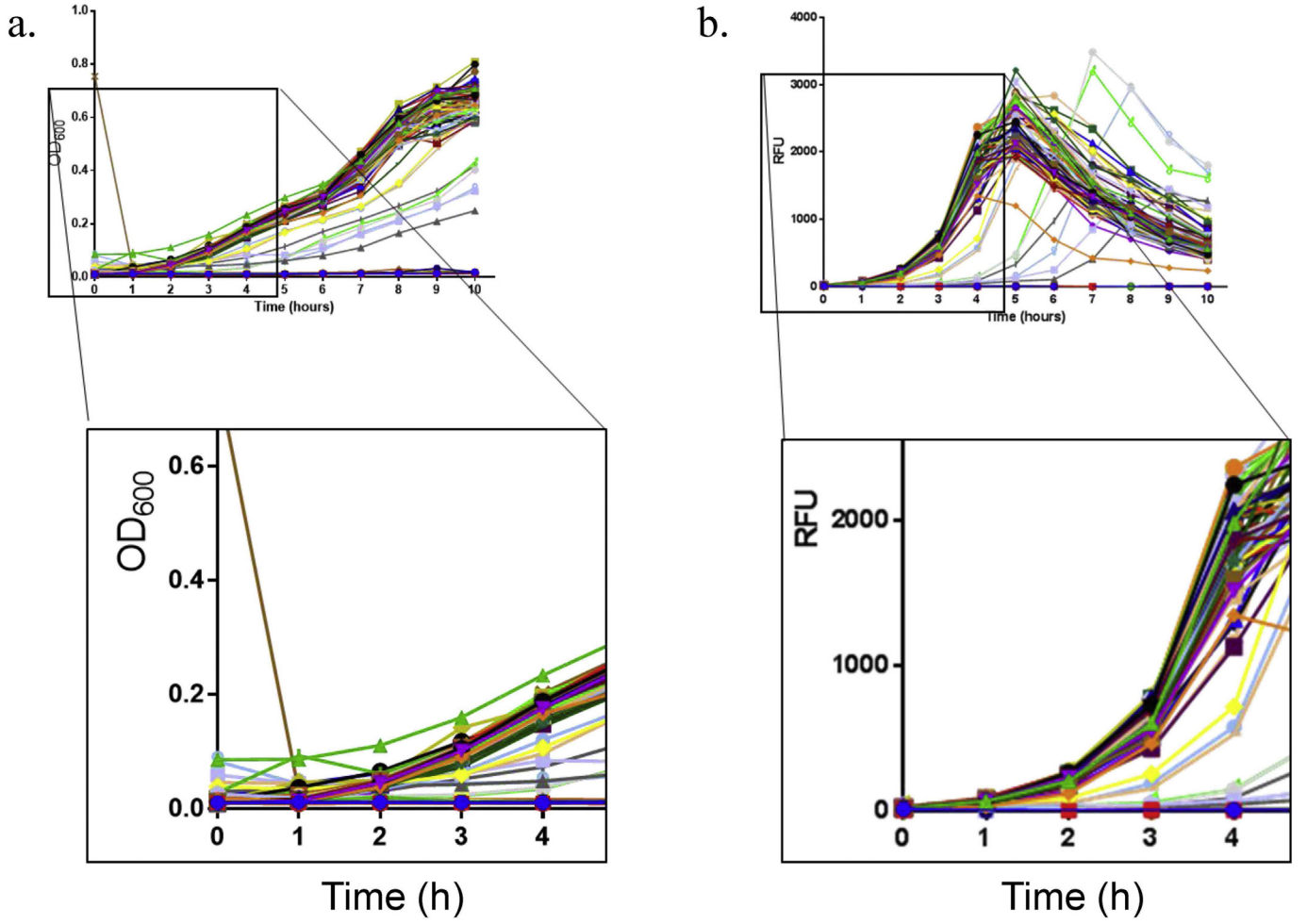

Fig. 1. A comparison of absorbance-(a) and the luminescence-based (b) methods in screening for antimicrobial(s) produced by fungal isolates. The graphs show same set of isolates that were screened for the production of antimicrobial(s) by the two methods using the reporter strain PAAK106.

natural products. Although, luminescent-based reporter strains have been described previously for screening of antimicrobials (Moir et al., 2008; Wallace et al., 2015), their use was limited to the targeting of specific pathways. The use of an efflux-deletion strain lacking five different RND efflux pumps as well as the outer membrane protein (OpmH) in combination with luminescence allows for the screening of compound(s) with much wider activity against the reporter strain. As shown in this study, use of such a hypersensitive strain not only significantly enhances our ability to screen for compounds with antimicrobial activity but also allows the screening process to be much faster than traditional methods.

2. Material and methods

2.1. Isolation of fungi

Samples were collected from various sites in Dauphin and Winnipeg (Manitoba, Canada) from different types of composts (house hold waste, animal waste, leaf litter, and composts including various combinations of plant debris, worm castings, and wood chips, five year old goat manure pile, five year old leaf compost pile) based on methods described by (Ottow, 1972; Malloch, 1981). Samples were taken from various layers of the compost pile/site. Matured composts are nutrient rich and thus harbour complex microbial communities and one would expect these sites to be very competitive environments with many microbes competing for resources and the microbes would be challenged with the variable temperature regimes a typical compost pile undergoes during its natural cycle of "composting" (Neher et al., 2013). Briefly 100 mg of soil/compost material was suspended in 10 mL of sterile H_2O; after approximately 2 h, a 50 μL aliquot of the soil suspension was plated onto corn meal agar medium (Becton Dickinson, Mississauga, ON, Canada; 17 g/L) supplemented with Rose Bengal (Sigma-Aldrich, Oakville, ON, Canada; 30 mg/L) and streptomycin (ThermoFisher, Burlington, ON, Canada; 30 mg/L) and chloramphenicol (ThermoFisher; 50 mg/L). Rose Bengal reduces the spreading growth of fungi preventing rapid growers from overgrowing fungi with slower growth rates (Ottow, 1972). The agar plates were prepared in triplicates and incubated at 20 °C, room temperature (~22 °C and daylight) and 35 °C. Fungal colonies were picked from these plates and transferred onto agar plates containing modified Malt/Yeast extract agar medium (20 g/L malt extract, 2 g/L yeast extract, 2 g/L peptone, and 15 g/L agar; all components were from Becton Dickinson, except the agar (from ThermoFisher). A total of 529 unique fungal isolates were recovered. For short term storage, fungi were maintained on agar plates. For long term storage, the fungi were maintained on modified Malt/Yeast extract agar slants by periodic transfer to fresh media and stored at 4 °C.

2.2. Identification of fungi at genus level

Fungal strains were partially identified (Genus level) in part based on morphological observation and in some instances by the utilization of rDNA internal transcribed spacer sequence (ITS) data (Visagie et al., 2014). Nucleic acids were extracted from fungi as described in Hausner et al. (Hausner et al., 1992) and various primers were utilized for the